1, 4, 13, 21, 22, 24, 30 & 31

What Is Claimed Is:

1. A method of producing an isolated coexpression vector capable of expressing an antibody molecule of predetermined specificity, which method comprises:

(a) synthesizing a V_H-coding gene library containing a plurality of different V_H-coding DNA homologs by:

(i) separating the strands of a repertoire of V_H-coding genes, said repertoire comprising double-stranded nucleic acids each containing a V_H-coding strand annealed to a complementary strand;

(ii) treating said separated strands, under conditions suitable for polymerase chain reaction amplification, with first and second primers, each of said first primers having a nucleotide sequence corresponding to a sequence conserved among said V_H-coding strands, and each of said second primers having a nucleotide sequence capable of hybridizing to a sequence conserved among said complementary strands, said primers being capable of priming the amplification of a plurality of different V_H-coding DNA homologs from said V_H-coding gene repertoire, said treating producing said V_H-coding gene library;

(b) synthesizing a V_L-coding gene library containing a plurality of different V_L-coding DNA homologs by:

(i) separating the strands of a repertoire of V_L-coding genes, said repertoire comprising double-stranded nucleic acids each containing a V_L-coding strand annealed to a complementary strand;

(ii) treating said separated strands, under conditions suitable for polymerase chain reaction amplification, with first and second primers, each of said first primers having a nucleotide sequence corresponding to

a sequence conserved among said V_L-coding strands, and each of said second primers having a nucleotide sequence corresponding to a sequence conserved among said complementary strands, said primers being capable of priming the amplification of a plurality of different V_L-coding DNA homologs from said V_L-coding gene repertoire, said heating producing said V_L-coding gene library;

(c) forming a diverse library of coexpression vectors by treating expression vector molecules adapted for ligation to the V_H- and V_L-coding DNA homologs of steps (a)(ii) and (b)(ii), respectively, with a diverse plurality of said V_H-coding DNA homologs and a diverse plurality of said V_L-coding DNA homologs, under conditions suitable for DNA ligation to produce a plurality of different coexpression vectors, each of said different coexpression vectors being capable of expressing an antibody molecule comprising a combination of V_H and V_L polypeptides that is different from the combination of V_H and V_L polypeptides forming antibody molecules expressed by any other of said different coexpression vectors; and

(d) segregating from said diverse library of coexpression vectors a coexpression vector capable of expressing an antibody of predetermined specificity.

2. The method of claim 1 wherein said expression vector molecules are linear DNA expression vector molecules.

3. The method of claim 2 wherein said linear DNA expression vector molecules are phage vector molecules.

4. A method of producing a V_H-coding gene library containing a plurality of different V_H-coding DNA homologs, which method comprises:

(a) separating the strands of a repertoire of V_H-coding genes, said repertoire comprising double-stranded nucleic acids each containing a V_H-coding strand annealed to a complementary strand; and

(b) treating said separated strands, under conditions suitable for polymerase chain reaction amplification, with first and second primers, each of said first primers having a nucleotide sequence corresponding to a sequence conserved among said V_H-coding strands, and each of said second primers having a nucleotide sequence capable of hybridizing to a sequence conserved among said complementary strands, said primers being capable of priming the amplification of a plurality of different V_H-coding DNA homologs from said V_H-coding gene repertoire, said treating producing said V_H-coding gene library.

5. The method of claim 4 wherein step (b) is performed using a plurality of different first primers.

6. The method of claim 4 wherein step (b) is performed using a plurality of different second primers.

7. The method of claim 6 wherein step (b) is performed using a plurality of different first primers.

8. The method of claim 4 further comprising multiple cycles of steps (a) through (b) using different first and/or second primers and combining the V_H-coding gene libraries produced by each cycle.

9. The method of claim 8 wherein each cycle is performed using a different repertoire of V_H-coding genes.

10. The method of claim 4 wherein said expression vector molecules are linear DNA expression vector molecules.

11. The method of claim 10 wherein said linear DNA expression vector molecules are phage vector molecules.

12. The method of claim 11 wherein said lambda phage vector molecules are Lambda Zap II V_H molecules.

13. A method of producing a V_L-coding gene library containing a plurality of different V_L-coding DNA homologs, which method comprises:

(a) separating the strands of a repertoire of V_L-coding genes, said repertoire comprising double-stranded nucleic acids each containing a V_L-coding strand

annealed to a complementary strand; and

(b) treating said separated strands, under conditions suitable for polymerase chain reaction amplification, with first and second primers, each of said first primers having a nucleotide sequence corresponding to a sequence conserved among said V_L-coding strands, and each of said second primers having a nucleotide sequence corresponding to a sequence conserved among said complementary strands, said primers being capable of priming the amplification of a plurality of different V_L-coding DNA homologs from said V_L-coding gene repertoire, said heating producing said V_L-coding gene library.

14. The method of claim 13 wherein step (b) is performed using a plurality of different first primers.

15. The method of claim 13 wherein step (b) is performed with a plurality of different second primers.

16. The method of claim 15 wherein step (b) is performed using a plurality of different first primers.

17. The method of claim 13 further comprising multiple cycles of steps (a) through (b) using different first and/or second primers and combining the V_L-coding gene libraries produced by each cycle.

18. The method of claim 17 wherein each cycle is performed using a different repertoire of V_L-coding genes.

19. The method of claim 13 wherein said expression vector molecules are linear DNA expression vector molecules.

20. The method of claim 13 wherein said linear DNA expression vector molecules are phage vector molecules.

21. A method of producing a gene library comprising a plurality of different coexpression vectors, each of said coexpression vectors being capable of expressing an antibody molecule comprising a combination of V_H and V_L polypeptides that is different from the combination of V_H and V_L polypeptides forming antibody

molecules expressed by any other of said coexpression vectors, which method comprises:

(a) synthesizing a V_H-coding gene library containing a plurality of different V_H-coding DNA homologs by:

(i) separating the strands of a repertoire of V_H-coding genes, said repertoire comprising double-stranded nucleic acids each containing a V_H-coding strand annealed to a complementary strand;

(ii) treating said separated strands, under conditions suitable for polymerase chain reaction amplification, with first and second primers, each of said first primers having a nucleotide sequence corresponding to a sequence conserved among said V_H-coding strands, and each of said second primers having a nucleotide sequence capable of hybridizing to a sequence conserved among said complementary strands, said primers being capable of priming the amplification of a plurality of different V_H-coding DNA homologs from said V_H-coding gene repertoire, said treating producing said V_H-coding gene library;

(b) synthesizing a V_L-coding gene library containing a plurality of different V_L-coding DNA homologs by:

(i) separating the strands of a repertoire of V_L-coding genes, said repertoire comprising double-stranded nucleic acids each containing a V_L-coding strand annealed to a complementary strand;

(ii) treating said separated strands, under conditions suitable for polymerase chain reaction amplification, with first and second primers, each of said first primers having a nucleotide sequence corresponding to a sequence conserved among said V_L-coding strands, and each of said second primers having a nucleotide sequence corresponding to a sequence conserved among said complementary strands, said primers being capable of priming

the amplification of a plurality of different V_L-coding DNA homologs from said V_L-coding gene repertoire, said heating producing said V_L-coding gene library;

(c) treating expression vector molecules adapted for ligation to the V_H- and V_L-coding DNA homologs of steps (a)(ii) and (b)(ii), respectively, with a diverse plurality of said V_H-coding DNA homologs and a diverse plurality of said V_L-coding DNA homologs, under conditions suitable for DNA ligation to produce a plurality of different coexpression vectors, each of said different coexpression vectors being capable of expressing an antibody molecule comprising a combination of V_H and V_L polypeptides that is different from the combination of V_H and V_L polypeptides forming antibody molecules expressed by any other of said different coexpression vectors, thereby forming said gene library.

22. A method of producing a monoclonal antibody molecule of predetermined specificity, which method comprises:

(a) synthesizing a V_H-coding gene library containing a plurality of different V_H-coding DNA homologs by:

(i) separating the strands of a repertoire of V_H-coding genes, said repertoire comprising double-stranded nucleic acids each containing a V_H-coding strand annealed to a complementary strand;

(ii) treating said separated strands, under conditions suitable for polymerase chain reaction amplification, with first and second primers, each of said first primers having a nucleotide sequence corresponding to a sequence conserved among said V_H-coding strands, and each of said second primers having a nucleotide sequence capable of hybridizing to a sequence conserved among said complementary strands, said primers being capable of priming the amplification of a plurality of different V_H-coding DNA

homologs from said V_H-coding gene repertoire, said treating producing said V_H-coding gene library;

(b) synthesizing a V_L-coding gene library containing a plurality of different V_L-coding DNA homologs by:

(i) separating the strands of a repertoire of V_L-coding genes, said repertoire comprising double-stranded nucleic acids each containing a V_L-coding strand annealed to a complementary strand;

(ii) treating said separated strands, under conditions suitable for polymerase chain reaction amplification, with first and second primers, each of said first primers having a nucleotide sequence corresponding to a sequence conserved among said V_L-coding strands, and each of said second primers having a nucleotide sequence corresponding to a sequence conserved among said complementary strands, said primers being capable of priming the amplification of a plurality of different V_L-coding DNA homologs from said V_L-coding gene repertoire, said heating producing said V_L-coding gene library;

(c) forming a diverse library of coexpression vectors by treating expression vector molecules adapted for ligation to the V_H- and V_L-coding DNA homologs of steps (a)(ii) and (b)(ii), respectively, with a diverse plurality of said V_H-coding DNA homologs and a diverse plurality of said V_L-coding DNA homologs, under conditions suitable for DNA ligation to produce a plurality of different coexpression vectors, each of said different coexpression vectors being capable of expressing an antibody molecule comprising a combination of V_H and V_L polypeptides that is different from the combination of V_H and V_L polypeptides forming antibody molecules expressed by any other of said different coexpression vectors;

(d) transforming a population of host cells compatible with said coexpression vectors with a plurality

of said different coexpression vectors to produce a transformed population;

(e) culturing said transformed population under conditions for expressing the antibody molecules coded for by said V_H- and V_L-coding DNA homologs;

(f) assaying the members of said transformed population for expression of an antibody molecule capable of binding a preselected ligand; thereby identifying a transformant capable of producing said monoclonal antibody; and

(h) harvesting from a monoclonal culture of said identified transformant of step (g) the antibody molecules produced by said culture, thereby producing said monoclonal antibody.

23. The method of claim 22 wherein said monoclonal antibody is catalytic.

24. A gene library comprising at least 10^5 different coexpression vectors, each of said coexpression vectors being capable of expressing an antibody molecule comprising a combination of V_H and V_L polypeptides that is different from the combination of V_H and V_L polypeptides forming antibody molecules expressed by any other of said coexpression vectors.

25. The gene library of claim 24 wherein each of said coexpression vectors comprise a V_H- and V_L-coding DNA homolog operatively linked for dicistronic expression to a linear DNA expression vector.

26. The gene library of claim 25 wherein said expression vector is lambda phage or a derivative thereof.

27. A V_H-coding gene library produced by the method of claim 4.

28. A V_L-coding gene library produced by the method of claim 13.

29. The gene library produced by the method of claim 21.

30. A gene library comprising at least 10^5 different V_H-coding DNA homologs, each of said homologs present as a population of DNA strands wherein the ratio of the number of said strands of a first length to the number of said strands having a length other than said first length is at least 4:1.

31. A gene library comprising at least 10^5 different V_L-coding DNA homologs, each of said homologs present as a population of DNA strands wherein the ratio of the number of said strands of a first length to the number of said strands having a length other than said first length is at least 4:1.

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